

WE CLAIM:

1. A biosensor comprising:
 - an optical grating;
 - 5 a substrate layer that supports the optical grating;
 - the optical grating being replicated from a master grating structure by causing a liquid to harden after dispensing the liquid between the master grating structure and the substrate layer, wherein the optical grating comprises the hardened liquid and wherein the optical grating comprises features having a depth and a period; and
 - 10 one or more specific binding substances immobilized on a surface of the optical grating opposite the substrate layer;
 - wherein, when the biosensor is illuminated a resonant grating effect is produced on the reflected radiation spectrum, and wherein the depth and period of the optical grating are less than the wavelength of the resonant grating effect.
- 15 2. The biosensor of claim 1, wherein a narrow band of optical wavelengths is reflected from the biosensor when the biosensor is illuminated with a broad band of optical wavelengths.
3. The biosensor of claim 1, wherein the substrate comprises plastic.
4. The biosensor of claim 1, wherein the liquid comprises optical cement.

5. The biosensor of claim 4, wherein the optical cement is hardened by exposing it to ultraviolet light.

6. The biosensor of claim 1, wherein the surface of the optical grating opposite the substrate layer is coated with a material having a high refractive index.

5 7. The biosensor of claim 6, wherein the material having a high refractive index is selected from the group consisting of zinc sulfide, titanium dioxide, tantalum oxide, and silicon nitride.

8. The biosensor of claim 1 further comprising a cover layer on the surface of the optical grating opposite the substrate layer, wherein the one or more
10 specific binding substances are immobilized on the surface of the cover layer opposite the optical grating.

9. The biosensor of claim 8, wherein the cover layer comprises a material that has a lower refractive index than the optical grating.

10. The biosensor of claim 9, wherein the cover layer comprises a material
15 selected from the group consisting of glass, epoxy, and plastic.

11. The biosensor of claim 1, wherein the optical grating comprises a repeating pattern having a period of about 0.01 microns to about 1 micron and a depth of about 0.01 microns to about 1 micron.

12. The biosensor of claim 1, wherein the one or more specific binding substances are arranged in an array of distinct locations.

13. The biosensor of claim 1, wherein the one or more specific binding substances are immobilized on the optical grating by physical adsorption or by
5 chemical binding.

14. The biosensor of claim 12, wherein each of the distinct locations defines a microarray spot of about 50-500 microns in diameter.

15. The biosensor of claim 1, wherein the one or more specific binding substances are bound to their binding partners.

10 16. The biosensor of claim 1, wherein the one or more specific binding substances are selected from the group consisting of protein solutions, peptide solutions, DNA solutions, RNA solutions, solutions of combinatorial chemical libraries, nucleic acids, polypeptides, antigens, polyclonal antibodies, monoclonal antibodies, single chain antibodies (scFv), F(ab) fragments, F(ab')₂ fragments, Fv
15 fragments, small organic molecules, cells, viruses, bacteria, and biological samples.

17. The biosensor of claim 16, wherein the biological sample is selected from the group consisting of blood, plasma, serum, gastrointestinal secretions, homogenates of tissues or tumors, synovial fluid, feces, saliva, sputum, cyst fluid, amniotic fluid, cerebrospinal fluid, peritoneal fluid, lung lavage fluid, semen,
20 lymphatic fluid, tears, and prostatitic fluid.

18. The biosensor of claim 15, wherein the binding partners are selected from the group consisting of proteins, peptides, single strand DNA, double strand DNA, RNA, chemical molecules in solution, nucleic acids, polypeptides, antigens, polyclonal antibodies, monoclonal antibodies, single chain antibodies (scFv), F(ab) fragments, F(ab')₂ fragments, Fv fragments, small organic molecules, cells, viruses, bacteria, and biological samples.

19. The biosensor of claim 18, wherein the biological sample is selected from the group consisting of blood, plasma, serum, gastrointestinal secretions, homogenates of tissues or tumors, synovial fluid, feces, saliva, sputum, cyst fluid, amniotic fluid, cerebrospinal fluid, peritoneal fluid, lung lavage fluid, semen, lymphatic fluid, tears, and prostatitic fluid.

20. The biosensor of claim 1, further comprising an antireflective dielectric coating on the surface of the substrate opposite of the two-dimensional grating.

21. The biosensor of claim 1, wherein the biosensor is attached to a bottomless microtiter plate by a method selected from the group consisting of adhesive attachment, ultrasonic welding and laser welding.

22. A liquid-containing vessel comprising the biosensor of claim 1 as an internal surface.

23. The liquid-containing vessel of claim 17, wherein the vessel is selected from the group consisting of a microtiter plate, a test tube, a petri dish and a microfluidic channel.

24. A detection system comprising the biosensor of claim 1;
5 a light source that directs light to the biosensor; and
a detector that detects light reflected from the biosensor, wherein a polarizing filter occurs between the light source and the biosensor.

25. A method of detecting the binding of one or more specific binding substances to their respective binding partners comprising:

- 10 (a) applying one or more binding partners to the biosensor of claim 1;
(b) illuminating the biosensor with light; and
(c) detecting a maxima in reflected wavelength, or a minima in transmitted wavelength of light from the biosensor;
wherein, if the one or more specific binding substances have bound to their
15 respective binding partners, then the reflected wavelength of light is shifted.

26. A method of detecting the binding of one or more specific binding substances to their respective binding partners comprising:

- (a) applying one or more binding partners to the biosensor of claim 1, wherein the optical grating is coated with an array of distinct locations containing the one
20 or more specific binding substances;
(b) illuminating each distinct location of the biosensor with light; and

(c) detecting maximum reflected wavelength or minimum transmitted wavelength of light from each distinct location of the biosensor;

wherein, if the one or more specific binding substances have bound to their respective binding partners at a distinct location, then the reflected wavelength of light is shifted.

27. A method of detecting activity of an enzyme comprising:

(a) applying one or more enzymes to the biosensor of claim 1;

(b) washing the biosensor;

(c) illuminating the biosensor with light; and

(d) detecting reflected wavelength of light from the biosensor;

wherein, if the one or more enzymes have altered the one or more specific binding substances of the biosensor by enzymatic activity, then the reflected wavelength of light is shifted.

28. A biosensor comprising:

an optical grating having a first and a second surface, the two-dimensional grating comprised of an optically transparent material that conducts electricity;

the optical grating being replicated from a master grating structure by causing a liquid to harden after dispensing the liquid between the master grating structure and a substrate layer, wherein the optical grating comprises the hardened liquid, and

wherein the second surface is in contact with the substrate layer, and wherein the optical grating comprises features having a depth and a period;

wherein the first surface of the optical grating is coated with an electrical insulator, wherein when the biosensor is illuminated a resonant grating effect is

produced on the reflected radiation spectrum, wherein the depth and the period of the optical grating are less than the wavelength of the resonant grating effect.

29. The biosensor of claim 28, wherein the optical grating is comprised of a repeating pattern of shapes selected from the group consisting of continuous parallel
5 lines, squares, circles, ellipses, triangles, ovals, trapezoids, sinusoidal waves, rectangles, and hexagons.

30. The biosensor of claim 28, wherein the repeating pattern of shapes are arranged in a linear grid, a rectangular grid or a hexagonal grid.

31. The biosensor of claim 28, wherein the optical grating has a period of
10 about 0.01 microns to about 1 micron and a depth of about 0.01 microns to about 1 micron.

32. The biosensor of claim 28, wherein two or more separate grating regions are present on the same substrate.

33. The biosensor of claim 32, further comprising an electrically
15 conducting trace to each separate grating region of the substrate.

34. The biosensor of claim 33, wherein the conducting trace is connected to a voltage source.

35. The biosensor of claim 32, wherein one or more specific binding substances are bound to each separate grating region of the substrate.

20 36. The biosensor of claim 35, wherein the one or more specific binding substances are bound to their respective binding partners.

37. A liquid-containing vessel comprising the biosensor of claim 28 as an internal surface.

38. The liquid-containing vessel of claim 37, wherein the vessel is selected from the group consisting of a microtiter plate, a test tube, a petri dish and a microfluidic channel.

39. A method of detecting the binding of one or more specific binding substances to their respective binding partners comprising:

- (a) applying one or more binding partners to the biosensor of claim 28;
 - (b) applying an electrical charge to the electrically conducting traces;
 - (c) illuminating the biosensor with light; and
 - (d) detecting reflected wavelength of light from the biosensor;
- wherein, if the one or more specific binding substances have bound to their respective binding partners, then the reflected wavelength of light is shifted.

40. The method of claim 39, further comprising the step of applying a reversed electrical charge to the electrically conducting traces before illuminating the biosensor with light.

41. A method of measuring the amount of one or more binding partners in a test sample comprising:

- (a) illuminating the biosensor of claims 1 or 28 with light;
- (b) detecting reflected wavelength of light from the biosensor;
- (a) applying a test sample comprising one or more binding partners to the biosensor;

(b) illuminating the biosensor with light; and

(c) detecting reflected wavelength of light from the biosensor;

wherein, the difference in wavelength of light in step (b) and step (f) is a measurement of the amount of one or more binding partners in the test sample.

5 42. A detection system comprising:

(a) the biosensor of claim 1;

(b) a laser source that directs a laser beam to a scanning mirror device, wherein the scanning mirror device is used to vary the laser beam's incident angle;

(c) an optical system for maintaining collimation of the incident laser beam; and

10 (d) a light detector.

43. The detection system of claim 42, wherein the scanning mirror device comprises a linear galvanometer.

44. The detection system of claim 43, wherein the linear galvanometer operates at a frequency of about 2 Hz to about 120 Hz and a mechanical scan angle of
15 about 10 degrees to about 20 degrees.

45. The detection system of claim 42, wherein the laser is a diode laser with a wavelength selected from the group consisting of 780 nm, 785 nm, 810 nm, and 830 nm.

46. A method of producing a biosensor comprising:
20 dispensing a liquid between a master grating structure and a substrate;

causing the liquid to harden, wherein the hardened liquid adheres to the substrate;

separating the substrate and the hardened liquid from the master structure, the hardened liquid replicating the master structure, the hardened liquid thus forming an optical grating that replicates the features of the master structure, the optical grating defining features having a depth and a period; and

immobilizing one or more specific binding substances on the optical grating;

wherein, when the biosensor is illuminated, a resonant grating effect is produced on the reflected radiation spectrum, and wherein the depth and period of the optical grating are less than the wavelength of the resonant grating effect.

47. The method of claim 46, further comprising:

depositing a coating onto the optical grating, the coating having a higher refractive index than the hardened liquid.

48. The method of claim 46, further comprising:

creating the master grating structure by selectively etching a silicon wafer to create optical features having a depth and a period.

49. The method of claim 46, wherein the liquid comprises optical cement.

50. The method of claim 49, wherein hardening the optical cement comprises exposure to UV light.

51. A method of producing a biosensor comprising:

creating a master grating structure by selectively etching a silicon wafer to create optical features having a depth and a period;

dispensing optical cement between the master grating structure and a
5 substrate;

curing the optical cement by exposing it to UV light, wherein the cured optical cement adheres to the substrate;

separating the substrate and the cured optical cement from the master grating structure, the cured optical cement replicating the master grating structure, the cured
10 optical cement thus forming an optical grating that replicates the features of the master grating structure, the optical grating defining features having a depth and a period;

coating the optical grating by sputter depositing a thin film of material selected from the group consisting of silicon nitride, titanium dioxide, zinc sulfide, or
15 tantalum oxide onto the optical grating; and

immobilizing one or more specific binding substances on the optical grating;

wherein, when the biosensor is illuminated, a resonant grating effect is produced on the reflected radiation spectrum, and wherein the depth and the period of the optical grating are less than the wavelength of the resonant grating effect.

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